



The role of human kallikrein 6, clusterin and adiponectin as potential blood biomarkers of dementia



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ARTICLE INFO

Article history:

Received 14 September 2015

Received in revised form 21 October 2015

Accepted 23 October 2015

Available online 26 October 2015

Keywords:

Dementia

Kallikrein 6

Adiponectin

Clusterin

ABSTRACT

Objectives: Progressive degenerative syndromes which affect brain, altering memory, behavior, cognition and emotion, are commonly defined as dementia. It was suggested that serum human kallikrein 6 (KLK6), clusterin (CLU) and adiponectin (ADPN) in combination with inflammation markers, neuroimaging and neuropsychological testing could assist in discriminating dementia patients from control individuals. Our aim was therefore to compare serum concentrations of KLK6, CLU and ADPN and inflammatory marker, interleukin-6 (IL-6), in patients suffering from Alzheimer's disease (AD), patients with vascular dementia (VAD), cognitively healthy participants (CHP) and those with mild cognitive impairment (MCI).

Design and methods: Serum samples were collected from AD, VAD and MCI patients admitted to the University Department of Neurology (Zagreb, Croatia) for regular follow-up. All patients underwent standard neuroimaging procedures including brain CT, neurosonological assessment with intima-media thickness (IMT) and breath holding index (BHI) calculations. Cognitive abilities were tested using standard Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Concentrations of KLK6, CLU, ADPN and IL-6 were determined in all serum samples.

Results: We have recruited a total of 235 participants, divided in 4 groups: AD (N = 70), VAD (N = 67), MCI (N = 48) and CHP (N = 50). Serum concentrations of KLK6 (P = 0.137), CLU (P = 0.178) and ADPN (P = 0.268) did not differ between AD, VAD, MCI and cognitively healthy control group of participants, whereas IL-6 was significantly higher in VAD patients than in AD, MCI and CHP individuals (P = 0.014). There was no association between investigated biomarkers and clinical patient parameters.

Conclusions: Serum concentrations of KLK6, CLU and ADPN do not differ between AD, VAD and controls with and without mild cognitive impairment. Higher IL-6 levels in VAD group point to the inflammatory component in the development of vascular dementia. Investigated biomarkers are not associated with neuroimaging findings and neuropsychological patient data.

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1. Introduction

Dementia is a common name for a group of progressive degenerative syndromes which substantially affect the brain and alter memory, behavior, thinking and emotion [1]. Early recognition and timely diagnosis of dementia or even mild cognitive decline is extremely important as it

enables opening a potentially larger therapeutic window. Alzheimer's disease (AD) is the most frequent cause of dementia. Clinical hallmark of AD is gradual loss of memory and cognitive capacity, resulting in individuals' inability to perform common everyday activities. Pathobiochemical features of AD are formation of amyloid plaques and neurofibrillary tangles in the hippocampus and entorhinal cortex of the brain [2]. Vascular dementia (VAD) is the second most common type of dementia, comprising 15 to 20% of all types of dementia [3,4]. VAD is a common consequence of cerebrovascular disease and vascular risk factors, especially hypertension and diabetes. Besides the memory decline, executive functions of the patients with vascular dementia are often impaired, and they also suffer from depression [5]. Since etiology of dementia may substantially

Abbreviations: AD, Alzheimer's disease; VAD, vascular dementia; MCI, mild cognitive impairment; CHP, cognitively healthy participants; CSF, cerebrospinal fluid; A β 42, amyloid β 42; KLK6, human kallikrein 6; CLU, clusterin; ADPN, adiponectin; IL-6, interleukin-6; IMT, intima-media thickness; BHI, breath holding index; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

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impact the patient management and outcome, early diagnosis of the type of dementia in its preclinical stage, is of vital importance. Mild cognitive impairment (MCI) is nowadays increasingly recognized as a disorder that often precedes clinically evident dementia [6]. MCI patients are not demented, but they have mild to minimal memory impairment that does not significantly impact daily functioning.

Recent studies have focused on neuroimaging and determination of amyloid- β 42 (A β 42) or tau-protein concentration in cerebrospinal fluid (CSF) as potential biomarkers for differentiating AD, VAD and MCI [7]. Due to invasive procedure of obtaining the CSF specimen, reliable blood biomarkers for early detection and follow-up of different forms of dementia are needed. Several biomarkers involved in the physiology of the brain tissue have been identified as potential candidates for early diagnosis and differentiation of dementia: human kallikrein 6 (KLK6), clusterin (CLU), adiponectin (ADPN) and inflammatory markers such as interleukin-6 (IL-6). The role of these blood biomarkers in the actual pathology is still controversial. KLK6, also known as neurosin, is serine protease involved in deposition of A β 42 peptide in brain tissue [8]. It has been shown that blood KLK6 concentration is affected by the advanced age and underlying neurologic pathology [9,10]. Clusterin (CLU) is a chaperone protein which is widely expressed in the brain tissue. Studies on its role in pathogenesis of AD showed that CLU can have opposite effects: cytoprotective effect by preventing the aggregation of A β 42, and exacerbating effect by enhancing the toxicity of A β oligomers [11]. Finally, adiponectin (ADPN) is adipocytokine produced by white adipose tissue which prevents development of cardiovascular diseases and type 2 diabetes. Recent studies indicate that ADPN affects insulin sensitivity, NO production and pro- and anti-inflammatory response in neurodegenerative disorders and VAD [12,13].

We hypothesized that, due to their different pathophysiological roles, serum concentrations of KLK6, CLU and ADPN could differ between subtypes of dementia. Former studies were focused on change in concentration of blood biomarkers in patient group with one diagnosis [9] or there was one blood biomarker assessed in wide range of neurological disorders [14]. Our aim was therefore to assess the difference in concentration of KLK6, CLU and ADPN between AD patients, VAD patients and control group consisted of the cognitively healthy individuals and those with MCI.

2. Materials and methods

2.1. Subjects

Serum samples were collected from AD and VAD patients who were admitted to the University Department of Neurology of the Medical School University Hospital Sestre milosrdnice (Zagreb, Croatia) for regular follow-up during the period from November of 2010 until the April of 2014. Diagnosis of AD was established based on clinical criteria and diagnostic guidelines, as described previously [15]. VAD was diagnosed based on recommendations of the Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke (NINDS) and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIRES) [16]. There were two control groups: a) control individuals with MCI and b) cognitively healthy individuals. Control individuals in both groups were older than 60 years. MCI was diagnosed according to well known Petersen's criteria [17]. Controls were recruited among spouses of AD and VAD patients included in the study and cognitively healthy patients referred to the University Department of Neurology by their general practitioners for the assessment of other neurological disorders.

2.2. Methods

For each study participant demographic (age, gender, body mass index (BMI), years of education completed during life) and vascular risk factor data (hypertension, diabetes mellitus, coronary disease, atrial

fibrillation, smoking) were collected. Current smokers and past smokers who have quit smoking within the last five years were classified as smokers, whereas non-smokers and past smokers who have quit smoking more than five years ago – were classified as non-smokers. A patient with systolic blood pressure of 140 mm Hg or higher and/or a diastolic blood pressure of 90 mm Hg or higher, and a patient currently treated with antihypertensive drugs were characterized as patient with hypertension [18]. If a patient was previously diagnosed with type I or type II diabetes, if he/she has at least two random glucose readings of >11.1 mmol/L or fasting blood glucose readings of >7.0 mmol/L, or he/she currently uses standard oral blood sugar lowering drugs or insulin, the patient is considered to have diabetes mellitus [19].

In all study participants, except in controls, brain multi-slice computed tomography (MSCT) was performed (Sensation Multislice Computed Tomography scanner with 16-row detector layer, Siemens, Germany) as a part of the routine diagnostic procedures. Brain MSCT findings were interpreted by neuroradiologist who was blinded to other patient data. Colour Doppler Flow Imaging (CDFI) of carotid arteries and measurement of intima-media thickness (IMT) and beta stiffness index (BSI) was performed on all study participants using the Aloka ProSound-5500 SSD PHD (Aloka Co.Ltd., Tokyo, Japan) with linear 10 MHz transducer according to well defined procedure and diagnostic protocol [20].

Additionally, transcranial Doppler sonography (using TCD Viasys Healthcare with 2 MHz probe, Madison, Wisconsin, USA) was performed in order to assess breath holding index (BHI) as a marker of cerebrovascular vasoreactivity. Increase of blood CO₂, provoked by holding of breath, results in vasodilatory reaction. Extent of reaction is measured by the BHI [21].

In all participants, Mini Mental State Exam (MMSE) and standardized Croatian version of Montreal Cognitive Assessment (MoCA) were used for cognitive assessment [22,23,24]. MMSE is usually used for evaluation of cognitive ability in population of patients with diagnosed dementia. Maximal score is 30 points, indicating absence of dementia, while score lower than 10 indicates severe dementia. MoCA is a test with high sensitivity for MCI with maximal score of 30 points and cut-off score of 26 points indicating MCI. Both tests can conveniently be used in clinical settings. Neurosonological measurements and cognitive testing were performed by experienced neurologists trained in vascular ultrasound and neuropsychological assessment.

Serum blood sample was collected from each study participant on admission. Regular biochemical tests included glucose, uric acid, cholesterol, HDL-cholesterol, LDL-cholesterol and CRP. Analysis was performed on the Beckman Coulter 2700 biochemistry analyzer (Beckman Coulter Inc., Brea, CA, USA) with original Beckman Coulter reagents (Beckman Coulter Biomedical Limited, Co Clare, Ireland). After routine analysis, leftover serum aliquots were immediately stored at –20 °C for KLK6, CLU, ADPN and IL-6 determination. Samples were handled with great care. Analysis was done after thawing. All samples were thawed only once.

Concentration of IL-6 was determined using the IL-6 reagent (Roche Diagnostics GmbH, Mannheim, Germany) on electrochemiluminescence immunochemistry analyzer Cobas e411 (Roche Diagnostics GmbH, Mannheim, Germany). Quality control for IL-6 was done with Preci Control Multimarker 1 and 2 (Roche Diagnostics GmbH, Mannheim, Germany). Data for imprecision (CV) of IL-6 assay are 1.4% at 38.3 pg/mL and 1.4% at 229 pg/mL concentrations.

Adiponectin testing was performed using the immunoturbidimetric assay ADPN (Adiponectin, Randox, Crumlin, United Kingdom) on biochemistry analyzer Beckman Coulter AU 680 (Beckman Coulter Inc., Brea, CA, USA). Quality control was done using Adiponectin Control – Level 2 (ADPN Control 2) (Randox Laboratories Limited, Crumlin, United Kingdom) and Adiponectin Control – Level 3 (ADPN Control 3) (Randox Laboratories Limited, Crumlin, United Kingdom). Within-laboratory CVs for ADPN assay were 2.0% at 4.0 μ g/mL and 3.4% at 9.6 μ g/mL concentrations.

Clusterin concentration was determined using CLU Human Clusterin ELISA (Bio Vendor Research and Diagnostic Products s.r.o., Brno, Czech Republic). Imprecision of the assay was 7.0% at 68.0 µg/mL, and 5.4% at 103.6 µg/mL.

Samples for KLK6 were shipped by express delivery service on dry ice to the Department of Pathology and Laboratory Medicine at Mount Sinai Hospital in Toronto, where its concentration was measured with KLK6 in-house ELISA method. The within-laboratory imprecision (CV) of the KLK6 assay is 5% at the range of 0.05–0.5 ng/mL of KLK6.

Ethical Board of Medical School University Hospital Sestre milosrdnice (Zagreb, Croatia) gave consent for the study performance and informed consents from all the participants were obtained.

2.3. Statistical analysis

Our study had 80% power (α of 0.05) to detect minimal differences of 0.9 ng/mL, 7.0 µg/mL and 3.3 µg/mL for KLK6, CLU and ADPN respectively. All the participant data were tested on normality by Kolmogorov–Smirnov test. Normally distributed data were represented as arithmetic mean and standard deviation. Data which do not follow normal distribution were represented as median and interquartile range (IQR), from first to third quartile.

Categorical data were tested with chi-square test. Quantitative data were tested with Kruskal–Wallis test when data did not follow normal distribution in at least one of the data sets. ANOVA one-way analysis of variance was used for data sets which followed normal distribution.

Spearman's coefficient of rank correlation (ρ) was used for non-Gaussian distributions and Pearson correlation for normally distributed variables. The level of statistical significance was set at ≤ 0.05 . Bonferroni correction was done for multiple testing (correlation analysis). For correlation analysis $P \leq 0.001$ was considered statistically significant.

Statistical analysis was performed using MedCalc Statistical Software version 13.3.3 (MedCalc statistical Software, Ostend, Belgium).

3. Results

Total number of participants was 245. Ten participants were excluded from the study based on: hemolytic serum ($N = 1$), insufficient

sample volume ($N = 2$), elevated CRP results (>32 mg/dL) ($N = 3$) and age (<60 years) ($N = 4$). Final number of included participants was 235.

In group of demographic data, statistically significant differences between the tested groups were found for age, BMI and education. Patients with VAD were oldest with median age of 78 years, while patients from control group of CHP were youngest with median age of 66 years. Patients with diagnosis of vascular dementia had lowest BMI, while other groups of participants had similar BMI values. Control group participants had higher number of years of education than patients with diagnosis of Alzheimer's disease and vascular dementia. Data on risk factors, hypertension, atrial fibrillation and smoking were found to be significantly different, showing the highest proportion of hypertension and atrial fibrillation in VAD patients. The largest proportion of smokers was found in CHP group. Routine biochemical tests showed significant difference only for uric acid and CRP. Highest concentration of uric acid was found in CHP group and lowest in patients with diagnosis of AD, while highest concentration of CRP was found in the group of CHP and lowest average concentration was in AD group. As expected, most prominent changes in IMT and BSI were found in VAD group, as well as lowest BHI (Table 1.).

For the inflammatory marker IL-6 statistically significant difference was found between tested participant groups. Patients with diagnosis of VAD had highest IL-6 concentration, while lowest concentration was found in control group of participants with MCI. P values for the tested potential blood biomarkers showed no difference between participants with diagnosis of AD and VAD in comparison with CHP or MCI individuals (Table 2). Statistically significant difference was not found for tested biomarkers after correction for age (KLK6 $P = 0.328$; ADPN $P = 0.497$; CLU $P = 0.253$) and gender (KLK6 $P = 0.263$; ADPN $P = 0.428$; CLU $P = 0.299$).

Out of all tested clinical, demographic and laboratory parameters, we have observed only the following correlations:

- ADPN correlated with glucose, uric acid and HDL-cholesterol (r values were -0.316 , -0.348 and 0.379 ($P < 0.001$)).
- IL-6 correlated with uric acid and HDL-cholesterol (r values were 0.323 and -0.275 ($P < 0.001$)).
- CRP correlated with uric acid (r value was 0.298 ($P < 0.001$)).

Table 1

Clinical and demographic data for study participants.

Group	AD (N = 70)	VAD (N = 67)	MCI (N = 48)	CHP (N = 50)	P*
Age (years) (median/range)	74 (69–79)	78 (74–80)	72 (68–76)	66 (63–73)	<0.001
Females (N/proportion)	43/0.61	40/0.60	31/0.65	30/0.60	0.321
BMI (kg/m ²)	26.3 (24.7–27.2)	24.8 (22.9–26.1)	26.3 (24.7–27.1)	26.5 (25.1–30.0)	<0.001
Education (years)	10 (8–12)	10 (8–12)	12 (10–13)	12 (12–15)	<0.001
Hypertension (N/proportion)	47/0.67	60/0.90	32/0.67	30/0.60	0.002
Diabetes mellitus (N/proportion)	5/0.07	9/0.13	7/0.15	6/0.12	0.570
Coronary disease (N/proportion)	7/0.10	11/0.16	1/0.02	9/0.18	0.052
Atrial fibrillation (N/proportion)	9/0.13	17/0.25	6/0.13	3/0.06	0.025
Smoking (N/proportion)	51/0.73	42/0.63	29/0.60	43/0.86	0.036
Glucose (mmol/L)	5.4 (5.1–6.1)	5.4 (4.9–6.2)	5.7 (5.1–6.9)	5.7 (5.2–6.2)	0.416
Uric acid (µmol/L)	274 (218–352)	299 (251–349)	291 (251–381)	334 (284–406)	0.011
Cholesterol (mmol/L)	5.8 ± 1.3	5.4 ± 1.3	5.4 ± 1.2	5.9 ± 1.4	0.060
Triglycerides (mmol/L)	1.4 (1.1–1.9)	1.4 (1.1–1.8)	1.4 (1.0–1.8)	1.4 (1.1–1.9)	0.698
HDL-cholesterol (mmol/L)	1.4 (1.1–1.7)	1.4 (1.2–1.6)	1.4 (1.2–1.7)	1.4 (1.2–1.6)	0.964
LDL-cholesterol (mmol/L)	3.6 ± 1.2	3.3 ± 1.2	3.3 ± 1.0	3.8 ± 1.1	0.066
CRP (mg/L)	1.3 (0.8–2.8)	2.2 (0.9–4.6)	1.8 (0.9–2.8)	2.5 (1.1–7.1)	0.027
IMT (mm)	1.0 (0.8–1.2)	1.2 (1.1–1.3)	1.2 (1.0–1.3)	0.9 (0.7–1.1)	<0.001
BSI (beta stiffness index)	11.7 (10.7–13.0)	12.6 (11.7–14.0)	11.5 (9.8–15.8)	8.5 (6.8–10.8)	<0.001
MMSE	18 (13–21)	19 (15–23)	27 (26–29)	30 (28–30)	<0.001
MoCA	13 (8–16)	15 (9–18)	22 (20–24)	29 (27–30)	<0.001
BHI %/s	1.21 (0.95–1.37)	0.89 (0.67–1.16)	1.21 (0.87–1.41)	1.40 (1.28–1.55)	<0.001

AD = Alzheimer's disease; VAD = vascular dementia; MCI = mild cognitive impairment; CHP = cognitively healthy participants.

BMI = body mass index; CRP = C-reactive protein; IMT = intima-media thickness; MMSE = Mini Mental State Exam; MoCA = Montreal Cognitive Assessment; BHI = breath holding index.

* Normally distributed data are represented as arithmetic mean and standard deviation and data which do not follow normal distribution are represented as median and interquartile range (IQR) from first to third quartile. Categorical data are analyzed with chi-square test. Quantitative data were tested using the Kruskal–Wallis test when data did not follow normal distribution in one of the data sets compared and with the ANOVA one-way analysis of variance if the data sets followed normal distribution. P values ≤ 0.05 are written in bold font.

Table 2
Inflammatory marker IL-6 and biomarkers in AD, VAD, MCI and CHP groups.

	AD (N = 70)	VAD (N = 67)	MCI (N = 48)	CHP (N = 50)	P*
IL-6 (pg/mL)	2.7 (1.9–5.0)	4.1 (2.1–6.4)	2.3 (1.5–4.0)	3.2 (1.9–4.5)	0.014
ADPN (μ g/mL)	10.6 (8.4–13.8)	10.3 (7.0–13.4)	10.4 (7.7–14.8)	8.8 (5.8–13.3)	0.268
CLU (μ g/mL)	42.2 \pm 15.0	37.0 \pm 14.7	41.8 \pm 16.3	40.5 \pm 12.8	0.178
KLK6 (ng/mL)	2.9 (2.3–3.4)	2.7 (2.3–3.3)	2.6 (2.1–2.9)	2.8 (2.3–3.3)	0.137

AD = Alzheimer's disease; VAD = vascular dementia; MCI = mild cognitive impairment; CHP = cognitively healthy participants.

IL-6 = interleukin-6; ADPN = adiponectin; CLU = clusterin; KLK6 = human kallikrein 6.

* Kruskal–Wallis test was used when data did not follow normal distribution in one of the compared data sets. ANOVA one-way analysis of variance was used if the data sets followed normal distribution. P values \leq 0.05 are written in bold font.

4. Discussion

With this study, we have tried to evaluate three potential blood biomarkers of dementia: KLK6, CLU and ADPN. Despite some previous encouraging reports, our results did not provide any evidence for the role of these blood biomarkers in discriminating types of dementia. Nevertheless, our results point to the significant role of inflammation in VAD group and not in other groups, as VAD group had highest IL-6 blood levels.

Both groups of patients, those with dementia (AD or VAD) and controls, showed variety of education levels. The level of education is routinely assessed in patients with dementia, as it is an important factor for the interpretation of cognitive testing results. Previous researches have shown that education–dementia relationship is quite complex, as some studies have suggested that lower education is associated with an increased risk for dementia while others have not found positive relationship [25]. Both MMSE and MoCA scores are known to vary not only by age, but also by education level, so population based norms were developed for both tests in order to enable comparison of cognitive scores among individuals with different education levels [26,27]. One point was routinely attributed to individuals with \leq 12 years of education, when screening with MoCA. Furthermore, a correction of +1 point for 10–12 years of education and +2 points for 4–9 years of education was applied. Cognitive scores in our subjects were corrected accordingly in order to adjust potential differences in the level of education.

The role of KLK6 in brain pathophysiology has been explored by several investigators over the past several years. KLK6 was found in amyloid plaques and neurofibrillary tangles of brain tissues in dementia patients [8]. Rovelet-Lecrux et al. found that gene coding KLK6, together with two other genes (SLC30A3 and FPR2), is involved in modification of A β metabolism [28]. Menendez-Gonzalez et al. reported differences in measured plasmatic concentrations of KLK6 between AD, VAD and control group [14]. We were not able to reproduce these findings in our clinical setting. The reason for inconsistency might be difference in study design and sample size, as well as in the method of selection of controls. Another possible discrepancy might be due to the effect of different blood additives or differences in KLK6 concentration in serum and plasma [29].

Peripheral blood concentrations of CLU and interleukin 6 receptor were suggested to be a useful tool in prediction of dementia [30]. Recent studies on potential blood biomarkers of AD have found the positive association of CLU concentration and rate of cognitive decline [31]. Thambisetty et al. investigated plasma CLU concentration in individuals with MCI and control subjects in relation to longitudinal changes in brain volume using neuroimaging study. The significant difference between MCI and control group in CLU concentration was not detected [32]. These data are consistent with our findings. In another study, Thambisetty et al. found that increased plasma CLU concentration was associated to faster decline in AD subjects, as well as tendency to develop amyloid deposits in control population [33].

ADPN has recently been suggested to be a potential biomarker of dementia. Higher ADPN concentrations were found to be associated with lower risk of vascular dementia, ameliorated cerebrovascular dysfunction and cognitive decline and reduced degree of neuroinflammation [13]. Also, the regulation of ADPN receptors was indicated as a potential key to prevention and treatment of VAD, so the need for more studies has been emphasized, in order to examine the role of adiponectin in vascular dementia. ADPN levels have so far been studied in CSF and in serum. However, the available data are still inconsistent. Higher levels of ADPN in CSF of patients suffering from AD (N = 27) compared to controls (N = 28) and individuals with MCI (N = 18) have been found by Une et al. [34]. Due to the small sample size, it is possible that the observed difference is due to chance only, so extended studies have to be conducted in order to confirm their observations. Khemka et al. have studied the difference in serum concentration of ADPN, insulin and leptin between AD patients and healthy controls. They found significant difference in insulin concentration between AD patients and healthy controls, and positive correlation between severity of AD and serum levels of insulin and ADPN [35]. Again, our data do not support these findings, and are in line with reports from some other authors, who also did not find evidence for a difference in concentration of ADPN in serum between patients suffering from an early AD and control group [36]. One of the reasons for contradictory study results could be in detection of different molecular forms of ADPN in blood (total ADPN or high molecular weight forms). Our assay has measured total ADPN concentration.

Due to relatively small sample size of our patient groups, possible cause for our (negative) results regarding KLK6, CLU and ADPN serum concentrations might be a probable overlap of VAD and AD pathologies in our patients. It has been shown that a substantial proportion of brains which meets the neuropathological criteria for AD, also demonstrates lesions typical for vascular pathology, such as cerebral amyloid angiopathy, microvascular degeneration and periventricular white matter lesions [37,38,39]. Although ApoE polymorphism has been known to elevate the risk for AD, our study unfortunately did not include the assessment of ApoE status in our population. We acknowledge this as a possible limitation to our study.

Neuroinflammatory response to formation of amyloid plaques and neurofibrillary tangles results in raised levels of inflammatory markers [40,41]. Comparing the levels of cytokines and chemokines in brain extracts of control and AD group Sokolova et al. have found that IL-6, IL-8 (interleukin-8) and MCP-1 (monocyte chemoattractant protein-1) were increased in AD group [42]. Recent studies showed that astrocytes could play a key role in neuroinflammatory response on injury caused by deposition of amyloid beta. Their protective role changes to destructive when they are exposed to prolonged activation. In such circumstances, cytokines and pro-inflammatory mediators, released by astrocytes, amplify neurodegeneration [43]. Some authors hypothesize that not only local deposition of A β 42 peptide, but also systemic and chronic inflammatory diseases induce production of pro-inflammatory cytokines and promote cognitive decline [44]. Our findings related

to inflammatory markers, CRP and IL-6 are consistent with previously published data and suggest the putative role of vascular inflammation in the ethiopathophysiology of vascular type of dementia. In older individuals, increased levels of pro-inflammatory cytokines and markers are considered normal, so that population usually shows signs of chronic “low grade inflammation” [45]. In patients with AD, inflammatory markers were shown to have inhibitory effect on neurotransmission [46].

One possible explanation for differences in the results reported by different authors could be due to the analytical differences between methods applied in biomarker measurement. Whereas Menendez-Gonzalez et al. have used commercial ELISA kit. For determination of KLK6 and CLU we have in our study measured KLK6 by the in-house method developed in the Department of Laboratory Medicine and Pathobiology at Mount Sinai Hospital (Toronto, Canada). Moreover, the differences could also be attributed to the differences in matrix. We have used serum in our study, while other authors have used plasma. It is possible that these issues might have contributed to some discrepancies among different studies.

In conclusion, although some previous reports have found the opposite, our data show no evidence for the differences in serum concentrations of KLK6, CLU and ADPN between AD, VAD and cognitively healthy controls and healthy controls with MCI. Difference between tested groups was found for the inflammatory marker IL-6. Investigated biomarkers, as suggested by our results, do not have a significant role in diagnosis and differential diagnosis of dementia. In order to confirm the validity of our results, they should be replicated in an independent patient population.

Acknowledgment

This study has been supported by the Ministry of Science, Education, and Sports, Republic of Croatia (project number: 134-1340227-0200).

We thank Randox Laboratories Ltd. for reagents, calibrators and control materials for adiponectin determination for this study.

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